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Recent Developments in the Mode of Action of Fungicides*

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Abstract: This review considers the effect of various fungicides, especially synthetic strobilurins, on fungal respiration, discusses recent contributions of biochemistry to the anilinopyrimidines and the phenylpyrroles and also discusses the future development of chemical inducers of systemic acquired resistance in host plants.

Key words: fungicide, mode of action, strobilurin, anilinopyrimidine, phenylpyrrole, plant activator.

1 INTRODUCTION

Effective fungal disease control strategies often require the use of fungicides. However, the chemical control of plant diseases presents disadvantages such as mammalian toxicity or the development of resistant strains. To face these problems there is a continuous need for new classes of antifungal agent. An important component of fungicide innovation is the identification of suitable and novel target sites. This review focuses on recent advances in the biochemical knowledge of respiratory inhibitors, on anilinopyrimidines and on phenylpyrroles. The search for activators of plant defences is also discussed. Recent reviews on the mode of action of fungicides can be found in References 1 and 2.

2 FUNGICIDES INTERFERING WITH RESPIRATION

The breakdown of organic molecules (i.e. sugars, fats, proteins) provides energy for the survival of living systems. In fungi (as in other eukaryotes), the final steps of this catabolic process take place in mitochondria and lead to the synthesis of the high energy intermediate ATP.

Several groups of fungicides disturb the energy supply in fungi and all such compounds are powerful inhibitors of spore germination. Among them, the oldest ones are non-specific thiol reactants such as copper derivatives, sulfur, dithiocarbamates (e.g. maneb, thiram) or 'R-S-CCl_3' compounds (e.g. captan, dichlofluanid). They inhibit several enzymes involved in respiratory processes, and this multisite action is believed to prevent the development of practical resistance to these protectant fungicides.[3] Recently, many representatives of this class of compound have come under increased toxicological scrutinity and some of these problems could be inherent in their non-selective reactivity.

Some of the fungicides which exhibit more specific effects on energy production directly prevent oxidative phosphorylation. They are either inhibitors, like the triphenyl-tin derivatives fentin acetate and fentin hydroxide or uncouplers like dinocap and fluazinam.[1,2] Fluazinam (Fig. 1), a phenylpyridinamine recently introduced in European countries, is active against potato blight (*Phytophthora infestans* (Mont.) de Bary).[4] It is a potent uncoupler in mammalian mitochondria but it is detoxified by glutathione.[5] Its mechanism of action is governed by the protonation/deprotonation of the amino group.[2,5]

Specific inhibitors of components of the mitochondrial respiratory chain have been used or tested as fungicides. Fenaminosulf was described as interfering with NADH-ubiquinone reductase (complex I),[3] but recent

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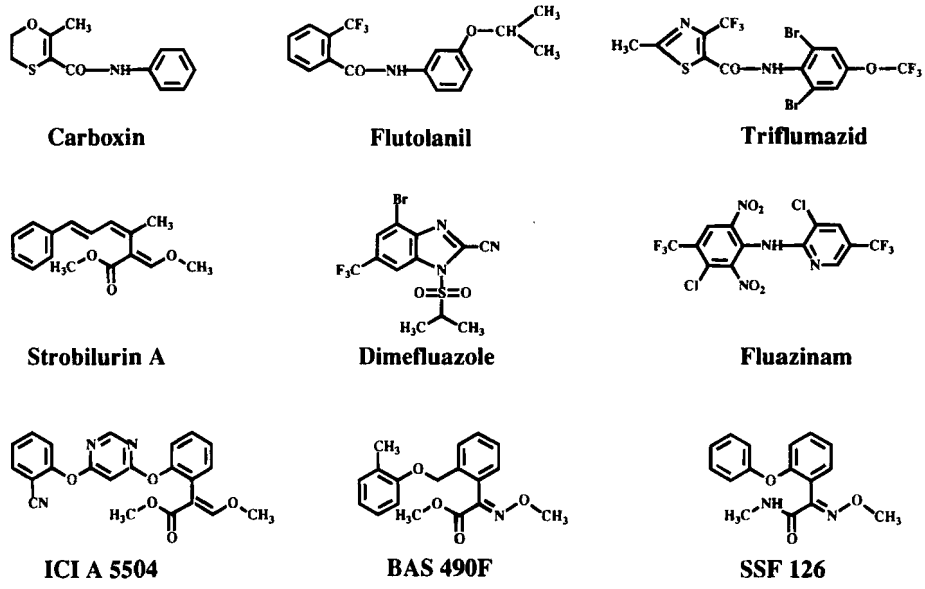


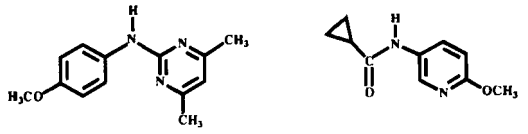
Fig. 1. Structures of fungicides interfering with respiration.

developments of this type of inhibitor have only produced miticides (e.g. fenazaquin, fenpyroximate, pyridaben, tebufenpyrad).[6] Thirty years ago the discovery of carboxin and its sulfone derivative oxycarboxin showed that succinate-ubiquinone reductase (complex II) was a target site for systemic fungicides.[2] Numerous other carboxamides have been developed; flutolanil and triflumazid are among the most recent ones (Fig. 1).[2,7] These carboxamides are effective *in vivo* in the control of fungal pathogens belonging to Basidiomycetes, but are also active when tested *in vitro* upon mitochrondria from Ascomycetes.[1,8] This observation suggests that their specificity is related to pharmacodynamics rather than target insensitivity, and that inhibitors of succinate-ubiquinone reductase with a broader spectrum could be found. The precise mechanism of action of these carboxamides is not totally understood. Photoaffinity-labelling studies indicate that their binding site is with the membrane-anchoring proteins (QPs or ubiquinone-binding proteins) of complex II and not to the two main subunits FP (a flavoprotein) and IP (an iron-sulfur protein).[1] On the other hand, in *Ustilago maydis* (DC.) Corda, a point mutation of the gene encoding the IP subunit confers resistance to carboxin.[9] From these findings, it can be hypothesized that carboxamides are intercalated between subunit IP and QPs in such a way as to hinder electron transfer to ubiquinone.[9]

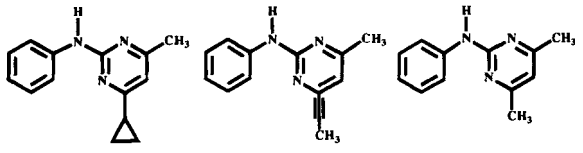
Several natural β-methoxyacrylates have been shown to interfere with ubiquinone-cytochrome c reductase or cytochrome b/c1 complex (complex III).[10] Among them, strobilurin A and oudemansin A are produced by the small fungi *Strobilurus tenacellus* (Pers. ex Fr.) Singer and *Oudemansiella mucida* Hoehn. Programmes of synthesis were initiated by BASF and ICI (many other companies have now published patent applications of β-methoxyacrylates and related compounds) in order to prepare analogues of the natural products exhibiting low acute mammalial toxicity, good light stability and systemic properties without any phytotoxicity. Several synthetic strobilurins are shown in Fig. 1; ICI A5504 is a methyl β-methoxyacrylate like strobilurin A whereas BAS 490F and SSF 126 are respectively a methyl methoxyiminoacetate and a methyl methoxyiminoacetamide.[11–13] BAS 490F and ICI A5504 are broad-spectrum fungicides controlling major Ascomycete, Basidiomycete and Oomycete plant pathogens on various crops;[11,12] they will probably be commercialised in 1996 or 1997. They strongly inhibit spore germination and consequently exhibit excellent protective activity; they have also eradicant properties towards apple scab and powdery mildews. The fungicidal activity of natural and synthetic strobilurins is a direct result of their ability to inhibit mitochondrial respiration.[13] Their selectivity seems to be based not on differences in mitochondrial target site, but on differences of penetration and degradation in various organisms.[2] The strobilurins bind to the ubiquinol oxidation centre of cytochrome b and consequently hinder the transfer of electrons from ubiquinol to cytochrome c.[14] Point mutations can lead to insensitivity of β-methoxyacrylates in yeasts (*Saccharomyces cerevisiae* Meyer ex Hanson).[15] Other compounds like the non-selective antibiotic antimycin A or the experimental anti-Oomycete dimefluazole (Fig. 1) are also known to interfere with the mitochondrial complex III. Both toxicants bind to the ubiquinone reduction centre of cytochrome b, which could be a novel target site for future agricultural fungicides.[16]

3 ANILINOPYRIMIDINES

Andoprim (Fig. 2), developed by VEB Fahlberg-List Magdeburg, was the first member of the anilinopyrimi-



andoprim SC-0858



cyprodinil mepanipyrim pyrimethanil

Fig. 2. Structures of fungicides interfering with methionine biosynthesis and/or enzyme secretion.

dine group. It is highly active against Peronosporales and it affects active transport processes across the cell membrane. This fungicide never reached the market.[2]

Three other anilinopyrmidines (without a methoxy group on the phenyl ring) have recently been introduced in European countries. They are mainly effective against diseases caused by Ascomycetes or Deuteromycetes. Mepanipyrim and pyrimethanil (Fig. 2) exhibit a high activity against grey mould and apple scab; cyprodinil (CGA 219417; Fig. 2) is also a promising fungicide for cereal diseases such as powdery mildew or eyespot.[17–19] These anilinopyrimidines do not affect spore germination but germ tube elongation is inhibited at low concentrations. Their *in-vitro* toxicity towards mycelial growth depends upon the medium and is greatly reduced in rich growth conditions. Several amino acids, particularly methionine, have been shown to reverse the fungitoxicity of the anilinopyrmidines.[20,21] Biochemical studies conducted in *Botrytis cinerea* Pers. Fr. with radio-labelled sulfate indicate that pyrimethanil inhibits the biosynthesis of methionine and suggest that the primary target could be cystathionine-β-lyase.[22] Similarities exist between these anilinopyrimidines and the experimental anilide SC 0858 (Fig. 2) whose fungitoxicity is also reversed by methionine.[23] In *Neurospora crassa*, Shear + Dodge, resistant mutants to SC 0858 are allelic to met 7, the gene encoding for cystathionine-γ-synthase.[24] However, enzymatic and biochemical studies have not shown any effect of SC 0858 on this enzyme and the methionine biosynthesis.[23]

Another common feature of mepanipyrim and pyrimethanil is their ability to prevent fungal secretion of hydrolytic enzymes such as protease, cellulase, lipase or cutinase which play an important role in the infection process.[25,26] In *B. cinerea*, the same phenomenon also concerns laccase and could explain the reduced laccase activity in grapes treated with pyrimethanil. The exact mechanism of action in the protein secretory pathway needs further investigation. However, the recent detection of strains of *B. cinerea* exhibiting *in-vitro* and *in-vivo* resistance to anilinopyrimidines suggests that these fungicides do not only interfere with pathogenic processes.[27]

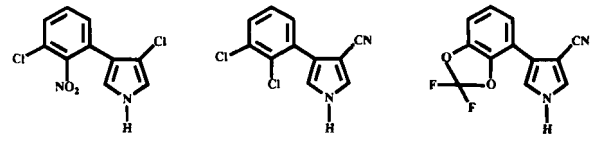
4 PHENYLPYRROLES

The phenylpyrrole antibiotic pyrrolnitrin is an antifungal compound produced by a number of *Pseudomonas spp.* and is thought to play a role in biocontrol activity by these bacteria.[28] Several synthetic analogues of pyrrolnitrin have been tested and two of them, fenpiclonil and fludioxonil (Fig. 3), have been developed as seed-dressing agents against numerous fungal species. Owing to its good light stability, fludioxonil can also be used as a foliar fungicide against *B. cinerea*, *Monilinia spp.* and *Sclerotinia spp.*[29,30]

The antifungal spectrum of activity of these phenylpyrroles is similar to that of dicarboximides (e.g. iprodione, procymidone, vinclozolin). In *B. cinerea*, they induce similar morphological alterations of germ tubes (i.e. swelling, branching, bursting) and their fungitoxicity is reversed by α-tocopherol and piperonyl butoxide.[31] Furthermore, in laboratory mutants of various fungal species, positive cross-resistance occurs between phenylpyrroles, dicarboxmides and aromatic hydrocarbons such as chloroneb, dichlobenil, dicloran or PCNB, as well as with tolclofos-methyl and etridiazol.[31,32] In *B. cinerea*, the gene Daf1 seems to be responsible for the resistance of laboratory mutants to both phenylpyrroles and dicarboximides.[33] Field isolates of *B. cinerea* with low resistance to dicarboximides were, however, normally sensitive to phenylpyrroles.[31–33]

In fungal mitochondria, pyrrolinitrin is able to uncouple oxidative phosphorylation and to inhibit electron transport. Such effects on respiration processes were also recorded for fenpiclonil, iprodione, tolclofos-methyl and etridiazol, but they occurred at concentrations 10 to 100 times greater than those which completely inhibited the mycelial growth.[31]

The antagonistic effect of the scavenger α-tocopherol reported in *B. cinerea* treated with fenpiclonil suggests that this fungicide stimulates the formation of reactive oxygen products, and as a consequence could induce peroxidation.[31] This effect, previously observed in several fungi treated by various dicarboximides or aromatic hydrocarbons, was considered to be the primary action of these fungicides. The target could be a plasma-



Pyrrolnitrin Fenpiclonil Fludioxonil

Fig. 3. Structures of phenylpyrrole fungicides.

membrane-bound NADPH-dependent flavin enzyme.[2] However, recent studies indicate that lipid peroxidation is not the primary mode of action of phenylpyrroles against *Fusarium sulphureum* (Schlecht) and of aromatic hydrocarbons or dicarboximides against *U. maydis*.[34,35] In *F. sulphureum*, the main metabolic modifications induced by fenpiclonil were an inhibition of wall glycan biosynthesis and an accumulation of neutral sugars. These effects indicate that the mechanism of action of fenpiclonil may be related to glucose metabolism. The target could be a transmembrane sugar carrier associated with glucose phosphorylation.[34] This mechanism of action of phenylpyrroles in fungi is close to that proposed for the herbicide dichlobenil (a member of the aromatic hydrocarbon group) in plants. According to Delmer *et al.*, dichlobenil might interfere with a membrane-bound protein involved in the regulation of the β-glucan synthesis.[36]

Of interest also are recent investigations conducted with laboratory mutants of *U. maydis* resistant to vinchlozolin and tolclofos-methyl. One gene conferring resistance was sequenced and compared to published sequences. There was high homology of the derived protein with serine (threonine) protein kinases.[37] In *S. cerevisiae*, protein kinases have been shown, to be associated with the cell cycle and cell-wall biogenesis.[38,39] Further work is needed to determine if the protein kinase is the direct target of the previous fungicides or if it is part of a resistance mechanism.

5 FUNGICIDES WITH AN INDIRECT MODE OF ACTION

Few non-fungitoxic compounds which regulate host resistance are presently commercialised. Possibly the only example is probenazole which controls rice blast (*Pyricularia oryzae* Bri. Cavara) and leaf blight (*Xanthomonas campestris* pv *oryzae*). This compound and saccharin, one of its major metabolites (Fig. 4), stimulate the production of fungitoxic lipids and of peroxidases in rice plants.[2] Similar involvement of host-defence mechanisms was also recorded in plants treated by the ambimobile fungicide fosetyl-Al and its main metabolite, phosphonic acid.[1,2,40] In fact, these phosphonates exert a direct effect on fungi (mainly Peronosporales) possibly by competing with phosphate.[41] Among the consequences of this direct action on fungal metabolism, an increase in elicitors or a decrease in suppressors of elicitor activity in plant tissue could occur.[1,2] Either effect would change a compatible (disease) response to an incompatible (no disease) response.

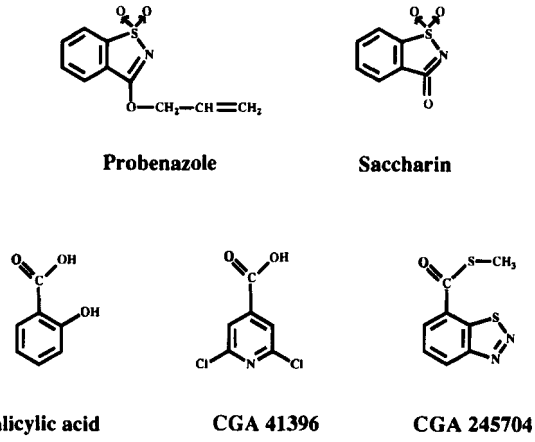
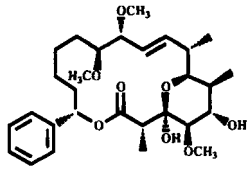


Fig. 4. Structures of antifungal agents with an indirect mode of action.

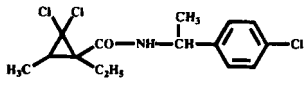
The most recent developments in the field of non-fungitoxic disease control compounds concern the discovery of functional analogues of salicylic acid.[42] Endogenous salicylic acid plays a crucial role in the induction of systemic acquired resistance (SAR). Derivatives of isonicotinic acid (CGA 41396) and of benzothiadiazole-7-carboxylic acid (CGA 245704) represent two classes of exogenous inducers of SAR (Fig. 4).[42–44] Treated plants can be protected from infection by fungal, bacterial and viral pathogens. In dicotyledonous plants, this acquired disease resistance correlates with the accumulation of pathogenesis-related (PR) enzymes such as proteinases, chitinases and peroxidases. These two classes of plant activators are believed to interact with the salicylic acid binding protein (receptor ?). Phytoxicity precluded the commercial development of isonicotinic acid derivatives but this drawback is not applicable to CGA 245704 when applied against wheat powdery mildew (*Erysiphe graminis*, D.C.), rice leaf blast (*P. oryzae*) or tobacco blue mould (*Peronospora tabacina* Adam). Because of a certain lag-time required for activation of the plant defence mechanisms, CGA 245704 has to be applied protectively or at an early stage in the disease progression.[44] Since the plant activators have no direct effects on fungi, the risk of development of fungal resistance is believed to be very low in comparison to fungicides with direct modes of action.

6 RECENT DEVELOPMENTS

Among the fungicides recently commercialised or in development, strobilurins and phenylpyrroles are synthetic analogues of natural products.[28] Strobilurins inhibit the mitochondrial respiratory chain, which confirms that respiration remains an attractive target for broad-spectrum fungicides. Recent investigations in the field of natural products have produced leads for new fungicides, e.g. soraphen A (Fig. 5), from the mycobacteria *Sorangium cellulosum* So. Soraphen A and its semi-synthetic analogues seem to be potential broad-spectrum fungicides. They are powerful inhibitors of acetyl-CoA-carboxylase from fungi and mammals.[43,45] This enzyme, which is involved in the first steps of *de-novo* fatty acid biosynthesis, is the primary target of



Soraphen A



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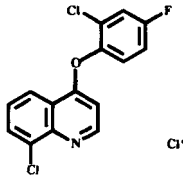

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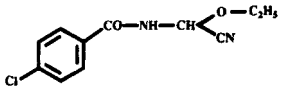
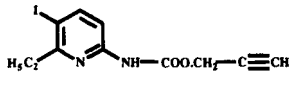


Zarilamide



Pyr. 1

Fig. 5. Structures of novel or experimental fungicides.

two classes of commercial grass-selective herbicides, the aryloxyphenoxypropionates (e.g. diclofop-methyl and quizalofop-ethyl) and the cyclohexanediones (e.g. sethoxydim and clethodim).[46] The practical success with these compounds should stimulate research for novel fungicides acting on acetyl-CoA-carboxylase.

The biochemical studies conducted on recent fungicides discovered in random screening resulted in the discovery of two original modes of action. The first case concerns the 8-chlorophenoxyquinoline LY 214352 (Fig. 5), which inhibits dihydro-orotate dehydrogenase, an enzyme involved in the biosynthesis of pyrimidines.[47] The second case concerns commercial anilinopyrimidines which inhibit methionine biosynthesis and which could be the first inhibitors of an amino acid biosynthesis pathway developed as fungicides. This type of mode of action is known for several major herbicides (e.g. glyphosate, glufosinate, sulfonylureas).[46]

Continuing research on known target systems concerns novel inhibitors of sterol biosynthesis, especially squalene epoxidase and lanosterol cyclase.[43] New antimicrotubular fungicides are also known, such as the anti-Oomycete zarilamide (Fig. 5) and experimental pyridylcarbamates e.g. Pyr. 1 (Fig. 5) which are effective against benzimidazole-sensitive and -resistant fungal strains.[48,49] More promising seems to be the novel melanin biosynthesis inhibitor KTU 3616 (proposed common name carpropamid; Fig. 5) which blocks the dehydration of scycalone.[50] This novel rice blast fungicide does not affect the reduction of 1,3,8-trihydroxynaphthalene which is the primary target site of older melanin biosynthesis inhibitors such as tricyclazole, pyroquilon and phthalide.[1,2]

7 CONCLUDING REMARKS

The main objective of biochemical studies on antifungal compounds discovered in random screening is to determine if they have an original mode of action.[43,51] In a second step, when the precise target site is known, *in-vitro* tests can be conducted for optimisation work.[43,52] In the future, the biorational design of new leads will probably be supported by advances in our understanding of fungal pathogenesis and molecular biology. The interest and the limitations of such an approach in agriculture as well as in medicine have recently been discussed.[1,43,52,53]

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